REMARKS

Prior to this response, claims 1-5 were pending. By the present communication, no claims have been added or cancelled and claims 1, 3 and 5 have been amended. The new claim language adds no new matter, being fully supported by the Specification and original claims. Accordingly, claims 1-5 are currently pending.

The Objection to Claim 3

Applicant respectfully traverses the objection to claim 3 for alleged lack of clarity of the phrase "in liquid phase assay" as used therein. The Examiner has provided no argument as to how those of skill in the art would be confused by the phrase at issue. However, to expedite prosecution and reduce the issues, Applicant has amended claim 3 to recite "in a liquid phase assay" as required by the Examiner. Accordingly, reconsideration and withdrawal of the objection to claim 3 are respectfully requested.

The Rejection under 35 U.S.C. § 112, Second Paragraph

Applicant respectfully traverses the rejection of claims 3-5 under 35 U.S.C. § 112, Second Paragraph, as allegedly being indefinite. With regard to claim 3, the Examiner asserts that the phrase "screening for a specified enzyme characteristic in a library of clones prepared by . . ." is confusing because it is unclear how recitation of how the clones will be used after their preparation in step (iii) can be a part of the preparation step. To overcome the rejection for alleged lack of clarity, step (iii) of claim 3 has been amended to recite "screening for the

specified enzymatic characteristic in an expression product prepared by . . . ," thereby eliminating any possible confusion regarding what is being prepared for screening.

The Examiner also asserts that the term "optionally" as used in claim 2 introduces lack of clarity regarding whether the limitation following the term is part of the claimed invention. To overcome the rejection, claim 2 has been amended to delete the term "optionally," thus overcoming the grounds for the rejection of claim 2.

Further with regard to claim 3, the Examiner asserts that the statement of the preamble is unclear because it allegedly is "unclear how a clone, which is an organism containing a specific DNA fragment, can display a protein characteristic" (Office Action, page 4). By the present communication, Applicant has amended the preamble to claim 3 to recite "A process of screening clones having DNA recovered from uncultivated organisms to identify an enzyme expressed therefrom having a specified enzymatic characteristic . . . , thus avoiding any possible confusion concerning how a clone displays a protein characteristic.

With regard to claims 3-5, Applicant disagrees with the Examiner's assertion that the claims are not limited to enzymes and are "still drawn to a method which screens for any protein characteristic" (Office Action, page 5). It is unclear to Applicants how an "enzymatic activity" can be discovered in any protein that is not an enzyme. However, in the interests of expediting prosecution and reducing the issues, by the present communication, claims 3-5 have been amended so as not to refer to an enzyme as a "protein" and to clarify that the "activity" produced by the enzyme is an "enzymatic activity." In particular, claim 5 has been amended to clarify that reference to "pH stability" and "temperature stability" means "pH stability of enzymatic activity" and "temperature stability of enzymatic activity."

In view of the amendments, Applicant respectfully submits that the invention screening methods, as defined by claims 1-5, meets all requirements under 35 U.S.C. § 112, second paragraph.

The Rejection under 35 U.S.C. § 112, First Paragraph – Written Description

Applicant respectfully traverses the rejection of claims 1-3 under 35 U.S.C. § 112, First Paragraph, for containing subject matter that allegedly is not adequately described in the Specification so that those of skill in the art would understand that Applicant had possession of the claimed invention at the filing of the application. Applicant disagrees with the Examiner's assertion that the Specification shows that the Applicant had described the invention in such a way that those of skill in the art would come to the conclusion that Applicant "had possession of the invention" only with respect to identifying *E. coli* clones comprising DNA isolated from a picoplankton sample wherein the clones express enzymes having hydrolase activity which are active after heating to 70° C for 45 minutes. Applicant further disagrees with the Examiner's assertion that the genome of the host cell would have to be known before those of skill in the art could determine whether the "desired characteristic" of enzymatic activity being produced was endogenously produced by the host cell or resulted from the "recovered DNA" placed into the host cell.

Given the knowledge of the art regarding enzyme activity tests (which the Examiner acknowledges is a large body), Applicant submits that those of skill in the art could easily determine whether every clone in the library was giving the same positive signal or displaying the same enzymatic characteristic, or whether one or more particular cells was producing a positive response that was *not common to the whole of the library* and hence attributable to the "recovered DNA." Once that determination is made and the responsible host cells noted, those of skill in the art would know how to obtain and further screen the product of the recovered DNA. Thus Applicant respectfully submits that a subtraction technique does not at all require use of a host cell whose complete genome is known, but can be accomplished simply by ignoring

or "subtracting out" as described in the Specification the enzymatic activities that are commonly produced by the host cells.

For example, Applicant respectfully submits that those of skill in the art do not need to be told the exact temperature or pH at which the host's enzymes cease to fully function. In a test for an enzyme that maintains stability of enzymatic activity at elevated or lowered pH or temperature, those of skill in the art would understand that the subtraction process consists simply of incrementally raising (or lowering) the pH or temperature of the expression products until enzymatic activity common to all of the host cells has been eliminated. Only specific expression products then remain (each attributable to the "recovered DNA" contained in a particular host cell) that maintain enzymatic activity at the extreme pH or temperature. Thus, in one aspect, the invention assay is designed to discover enzymes encoded by uncultivated organisms whose activity is maintained in the face of the extreme condition to which the library products are submitted. Accordingly, false positives produced in common by the host cells are easily eliminated in a library setting in a type of "subtraction" procedure simply by increasing the extremity of the condition on the expression products beyond that at which the host cell's enzymes (which are common to all of the clones) are active, by analogy with Applicant's description in the Specification of heating *E. coli* to 70 °C to inactivate host enzymes.

Accordingly, in view of the above amendments and arguments, Applicant respectfully submits that those of skill in the art would understand that the invention as described and as presently claimed was fully contemplated at the filing of the application.

The Rejection under 35 U.S.C. § 112, First Paragraph – Enablement

Applicant respectfully traverses the rejection of claims 1-5 under 35 U.S.C. § 112, first paragraph as allegedly lacking an enabling disclosure for the full scope of the claims. Applicant disagrees with the Examiner's assertion that claims 3-5 as previously amended would encompass any type of protein. For example, the Examiner asserts that claim 3 lacks enablement because the claim scope encompasses *any type* of protein characteristic. As previously amended, claim 3 clearly requires identification of a "specified enzymatic characteristic." Only enzymes have enzymatic characteristics. However, by the present communication, claims 3-5 have been further amended to underscore that the subject matter of the claims and the type of "characteristic" being assayed is limited to enzymes and enzymatic activities. In view of the above amendments, there should now be no question on the part of the Examiner that claims 1-5 pertain solely to enzymes and are not drawn to "any protein."

Applicant's remarks above concerning the description of the invention pertain equally and are incorporated here with respect to enablement.

Particularly with respect to claims 4 and 5, it is believed that those of skill in the art would consider as "routine" the testing for enzymatic activity to discover enzymes having stability of enzymatic activity under extreme conditions, such as elevated or lowered pH or temperature. No special skill is required to accomplish such screenings and the test for "undue experimentation" is whether an unusual degree of skill or inventiveness is required, not whether the testing is repetitive and/or routine. Accordingly, Applicants respectfully submit that claims 1-5 are fully enabled by the description of the Specification, and reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph, for allegedly lacking enablement are respectfully requested.

The Rejection under 35 U.S.C. § 103(a)

Applicant respectfully traverses the rejection of claims 1-5 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Yen et al. (U.S. Patent No. 5,171,684; hereinafter "Yen") in view of More et al. (Appl. Environ. Microbiol. 60(5):1572-1580, 1994; hereinafter "More"). The invention methods for identifying clones of a recombinant library produced from DNA derived from two or more uncultivated organisms which express an enzyme with a desired characteristic, as defined by amended claim 1, distinguish over the combined disclosures of Yen and More at least by requiring: "screening in the liquid phase a library of expression clones randomly produced from DNA of two or more uncultivated organisms, said screening being effected on expression products of said clones to thereby identify clones which express an enzyme with a desired characteristic. The invention methods, as defined by amended claim 3, distinguish over the combined disclosures of the cited art by requiring:

(i) recovering DNA selectively from a DNA population derived from two or more uncultivated organisms by contacting the recovered DNA in a liquid phase assay under hybridizing conditions with at least one hybridizing probe containing a full-length coding region sequence or a partial coding region sequence for an enzyme having the specified enzymatic characteristic;

(ii) transforming a host cell with the recovered DNA to produce a library of clones; and

(iii) screening for a specified enzymatic characteristic in an expression product prepared by expressing the library of clones to obtain expression products, which are screened to identify the specified enzymatic characteristic.

Applicant respectfully submits that Yen fails to suggest the invention method of claims 1 and 3 because in Yen's method the isolated DNA was obtained from a single cultured organism whose enzymatic products were known, and the DNA was pretreated so as to bias the DNA towards a particular known enzyme with a restriction endonuclease whose active site was known to exist in some or all of the genes encoding the predetermined target enzyme. Thus, Yen fails to suggest creating and screening a DNA library that is produced from the DNA of two or more uncultivated organisms (as required by claims 1 and 3), or that is "randomly produced from DNA of two or more uncultivated organisms" (as required by claim 1). In fact, the Examiner has completely overlooked the term "randomly produced" in claim 1, which signifies that the invention methods proceed in a manner that is exactly opposite to the method of library preparation followed by Yen, who obtains all of the DNA of a single cultivated organism and then goes through a series of steps designed to assure that the library includes only members that contain PmKR1 toluene monooxygenase genes (as described in the "Conjugation and Complementation and Screening Assay" of Example 3 of Yen).

In addition, Applicant submits that the invention methods of claims 4 and 5 are not suggested by Yen, and there is no suggestion in Yen that would motivate those of skill in the art to arrive at Applicant's invention. Yen discloses that the Pm KR-1 cells were mutagenized to obtain mutants defective in the enzyme p-cresol hydroxylase or p-hydroxybenzaldehyde dehydrogenase of the TMO pathway for use as the recipient strain in the conjugation assay (Col 9, lines 50-60). Thus, Yen fails to suggest or motivate those of skill in the art to mutagenize DNA recovered from a mixed population of organisms for formation of a library to be screened for identifying a mutant DNA encoding an enzyme with a "specified enzymatic characteristic" or having increased pH or temperature stability, as is the case in Applicant's invention as defined by claim 4.

Applicant respectfully submits that More does not cure these deficiencies in Yen so as to render obvious the invention methods. More's disclosure regarding isolation of DNA from an SDS-treated sediment sample containing a mixed population of organisms dwell on failures rather than successes. More focuses discussion on determining the most efficient method for isolation and purification of such DNA and concludes that even the most efficient method (bead mill homogenization) leaves approximately a portion of the organisms unlysed. PCR purification of a single predetermined gene, *nahR*, encoding the regulatory gene for naphthalene catabolism in Pseudomonas putida G7 (which *does not* encode an enzyme) was conducted only for the purpose of determining the performance efficiency of DNA extraction procedures being tested. More concluded that "the sediment contained PCR-inhibitory substances whose removal required a gel-electrophoretic purification step" (Page 1576, Col. 1, bottom). After further testing, More concluded that "the electrophoresis and SpinBind purification steps failed to completely remove substances inhibitory to the PCR" (page 1576, Col. 2, last full paragraph), so the combination with gel electrophoresis proved not to remedy the identified problem. In addition, More notes "inconsistency" in results obtained with various samples, an inconsistency noted by others who had attempted amplification of *nahAc* from upgradient and downgradient samples. More concludes by opining that even if the extraction efficiency were improved to 99.9%, there would still be 10^6 cells per gram of sediment whose DNA could not be accessed by current methods (page 1578, Col. 2). Thus, it must be admitted that More's comments regarding the utility and reliability of procedures for isolation and purification of DNA from a mixed population of uncultivated organisms would not be considered encouraging by those of skill in the art. Particularly, those of skill in the art would not be motivated by More's analysis of the limitations of isolation and purification procedures to develop an assay as disclosed by Yen in which the DNA of a mixed population of uncultivated organisms is substituted *randomly* for the DNA of a

single known and cultivated organism to produce a DNA expression library that is screened to identify clones encoding a desired enzymatic activity.

In addition, due to More's findings regarding the inefficiency and lack of reproducibility inherent in the methods used when an environmental sample is involved, even if those of skill in the art were motivated to combine the disclosures of Yen and More so as to arrive at the invention methods as presently claimed, Applicant respectfully submits that those of skill in the art would not have a reasonable expectation of success in identifying clones with DNA encoding an enzyme having a desired enzymatic characteristic.

Accordingly, Applicants submit that *prima facie* obviousness of claims 1-5 is not established over the combined disclosures of Yen and More and reconsideration and withdrawal of the rejection are respectfully requested.

The Terminal Disclaimer

The Office Action indicates that the Terminal Disclaimer submitted with the Response to the previous Office Action was directed to a different application having Serial Number 09/467,740. To remedy the error, Applicant submits the Terminal Disclaimer for the above-identified application to overcome the following rejections under the judicially created doctrine of obviousness-type double patenting raised in the previous Office Action mailed December 27, 2002:

Applicant respectfully traverses the provisional rejection of claims 1-3 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-22 of U. S. Patent No. 6,280,926 in view of More.

Applicant respectfully traverses the provisional rejection of claims 1-3 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-9 of U. S. Patent No. 6,168,919 in view of More.

Applicant respectfully traverses the provisional rejection of claims 1-3 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-15 of U.S. Patent No. 5,958,672 in view of More.

Applicant respectfully traverses the provisional rejection of claims 1-3 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 32-47 of copending application Serial No. 09/421,629 in view of More.

Applicant respectfully traverses the provisional rejection of claims 1-3 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 11, 14, and 16 of copending application Serial No. 09/467,740 in view of More.

Applicant respectfully traverses the provisional rejection of claims 1-3 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-20, 23-25, and 27-28 of copending application Serial No. 09/713,176 in view of More.

Applicant respectfully traverses the provisional rejection of claims 1-3 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-5 of copending application Serial No. 09/861,267 in view of More.

Applicant respectfully traverses the provisional rejection of claims 1-3 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-22 of copending application No. Serial 09/875,412 in view of More.

In traversal of the above provisional rejections for alleged double patenting, Applicants submit herewith a Terminal Disclaimer disclaiming the terminal part of any patent that may issue on claims of the present application that would extend beyond expiration of any of the above issued patents and patents that may issue from the above co-pending patent applications. In addition, Applicant submits that the present application and all of the co-pending applications and issued patents referenced in the Terminal Disclaimer were co-owned by Diversa Corporation

at the filing date of the present application. In view of the Terminal Disclaimer submitted herewith, Applicant respectfully submits that all of the patent applications and issued patents referenced in the terminal disclaimer are not available as prior art against the present application.

In addition, Applicant respectfully submits that the teachings of More alone are not sufficient to render unpatentable the subject matter of the invention methods, as defined by present claims 1-5. Although More describes methods for isolation of DNA from a sediment sample containing a mixed population of organisms, More amplifies a single predetermined gene, *nahR*, encoding the regulatory gene for naphthalene catabolism *in Pseudomonas putida* G7 and only for the purpose of determining the performance efficiency of the DNA extraction procedures tested by More. More does not prepare a library for screening or screen such a library for any type of molecule or activity. Thus, More fails to suggest how a library from an uncultured organism or a mixed population of uncultured organisms would be prepared for screening to identify unknown DNAs encoding proteins having a desired enzymatic property. In light of the Terminal Disclaimer and above remarks concerning More's deficiency for disclosing the present invention, Applicants respectfully request reconsideration and withdrawal of the double patenting rejection for alleged obviousness-type double patenting.

In view of the Terminal Disclaimer and above amendments and remarks, Applicant respectfully submits that all claims are now in condition for allowance, which is respectfully

requested. If the Examiner would like to discuss any issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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